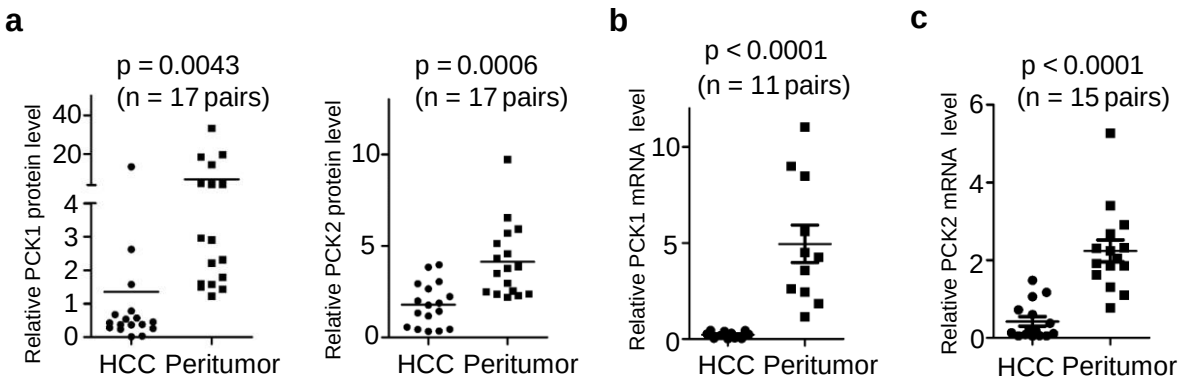
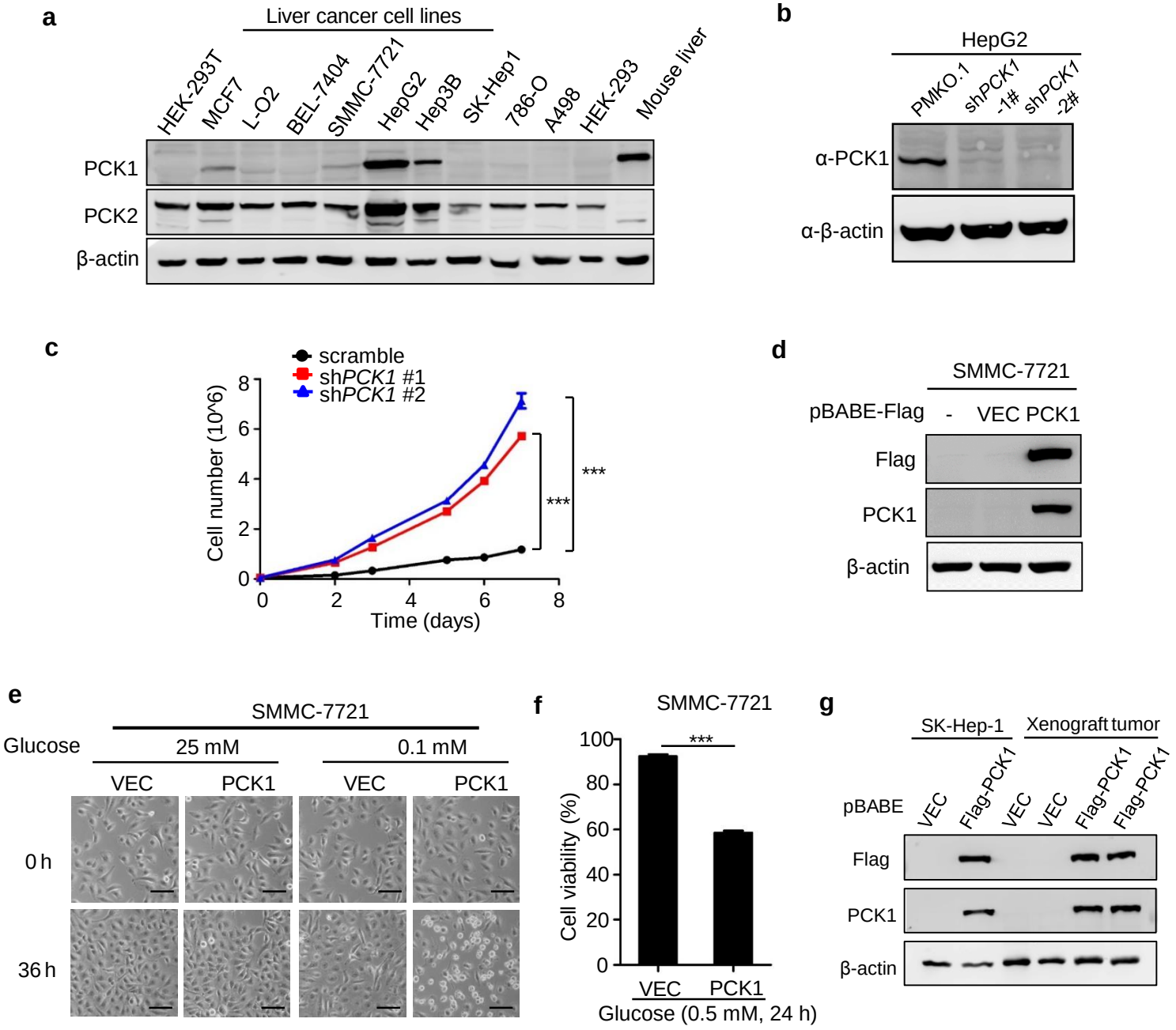
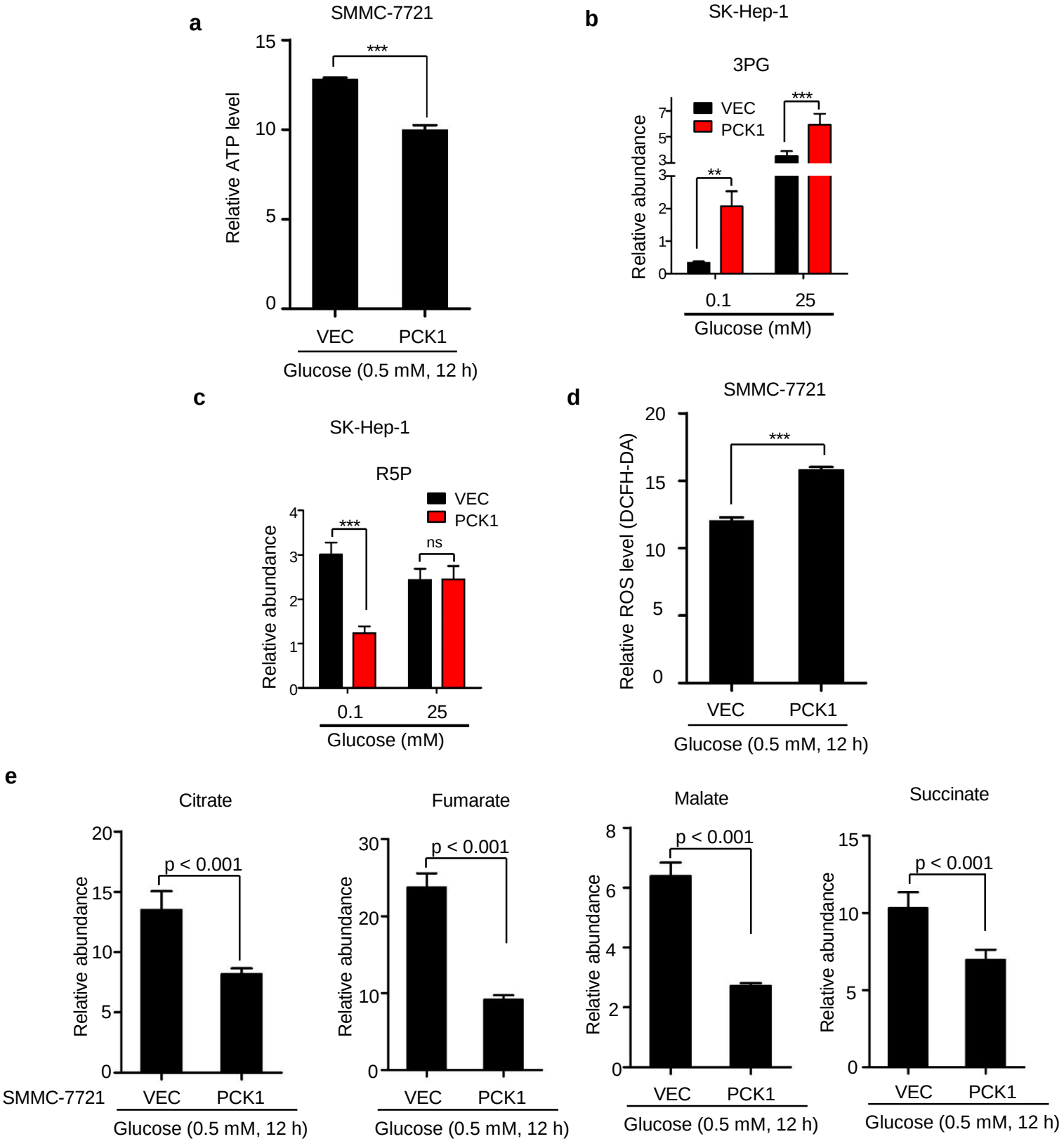


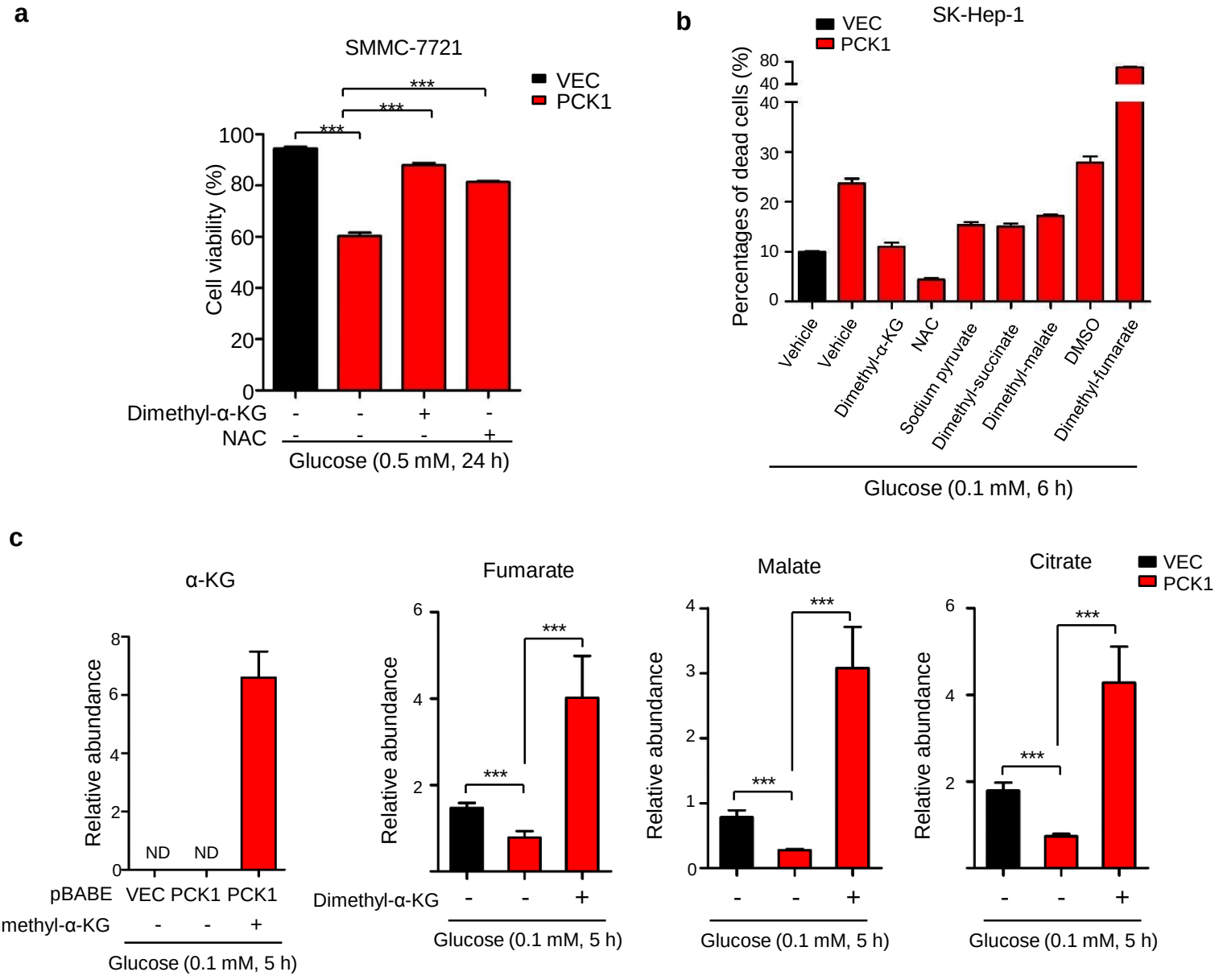
Supplementary Figure S1. PCK1 and PCK2 were downregulated in human HCC. (a) PCK1 and PCK2 protein levels in Figure 1g were normalized against β-actin and quantified using ImageQuant TL software, followed by statistical analysis. The horizontal bars represent mean PCK1 or PCK2 protein levels of 17 pairs of human liver tumors and adjacent normal liver tissues (**$p < 0.01$, ***$p < 0.001$). (b and c) Statistical analysis of PCK1 (b) and PCK2 (c) mRNA levels in Figure 1h and 1i, respectively. Results are mean PCK1 (b) or PCK2 (c) mRNA levels ± SD of 11 (b) or 15 (c) pairs of human liver tumors and adjacent normal liver tissues (***$p < 0.001$).



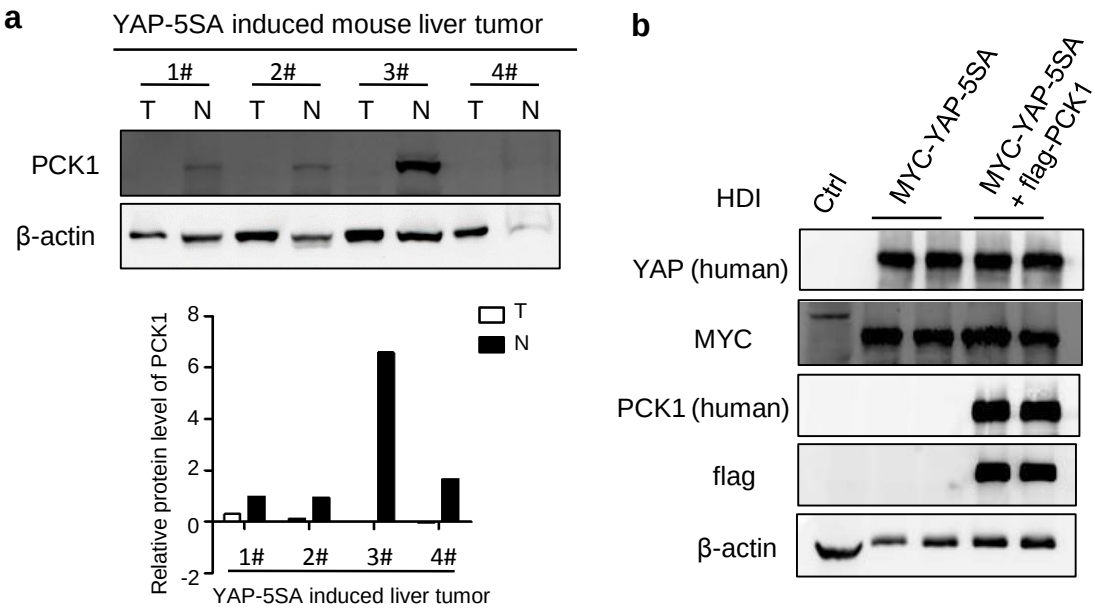
Supplementary Figure S2. Elevated expression of PCK1 in liver cancer cell lines caused cell death upon glucose deprivation. (a) Expression levels of PCK1 and PCK2 in a collection of liver derived cancer cell lines. (b) The knocking down efficiency of PCK1 in HepG2 cells were determined by Western blot. (c) Knocking down of PCK1 in HepG2 cells promoted cell proliferation cultured in media containing 5 mM of glucose. Results are mean cell numbers ± SD of triplicate samples. (d) Verification of SMMC-7721 cells that stably express flag-PCK1. (e) Ectopic expression of PCK1 in SMMC-7721 cells promoted cell death under low glucose condition (0.1 mM) for 36 h. Photos were taken under a phase-contrast microscope. Scale bars: 100 μm. (f) Cell viability of SMMC-7721 cells were determined by propidium iodide (PI) and Annexin V FITC staining after cultured in 0.5 mM glucose for 24 h. Results are mean cell viability ± SD of triplicate samples (***$p < 0.001$). (g) Verification of PCK1 expression in xenograft tumors by Western blotting.



Supplementary Figure S3. PCK1 induced TCA cataplerosis, energy crisis and oxidative stress in liver cancer cells. (a) Ectopic expression of PCK1 in SMMC-7721 decreased cellular ATP level in glucose starved cells. (b and c) SK-Hep-1 cells expressing empty vector or PCK1 were cultured in media containing 0.1 mM or 25 mM of glucose for 5 h. Cellular levels of 3PG and R5P were measured by LC-MS. Values represent mean abundance of indicated metabolites ± SD of quintuplicate samples (**$p < 0.01$; ***$p < 0.001$; ns, not significant). (d) Ectopic expression of PCK1 in SMMC-7721 resulted in higher ROS level. SMMC-7721 cells expressing empty vector or PCK1 were cultured in media containing 0.5 mM of glucose for 12 h. ROS level was detected by DCFH-DA staining as described in Methods. Values represent mean ROS level ± SD of triplicate samples (***p < 0.001). (e) Relative abundance of citrate, fumarate, malate and succinate was significantly decreased in glucose-starved SMMC-7721 cells ectopically expressing PCK1.



Supplementary Figure S4. ROS suppression or α-KG replenishment completely blocked the death of liver cancer cells ectopically expressing PCK1 upon glucose deprivation. (a) Dimethyl-α-KG and NAC significantly blocked PCK1 induced cell apoptosis in SMMC-7721 cells. Cell viability was determined by PI and annexin V staining. (b) Dimethyl-α-KG and NAC are most efficient in blocking PCK1 induced cell death among multiple TCA cycle-related metabolites. SK-Hep-1 cells expressing empty vector or PCK1 were deprived for glucose for 6 h in the absence or presence of dimethyl-α-KG (5 mM), NAC (10 mM), dimethyl-succinate (5 mM), dimethyl-malate (5 mM), dimethyl-fumarate (20 μM), sodium pyruvate (1 mM) and DMSO (0.1% V/V). Dead cells were detected by PI staining and analyzed by flow cytometry. Values represent mean percentages of dead cells ± SD of triplicate samples. (c) Supplement of dimethyl-α-KG significantly increased the relative abundance of TCA cycle intermediates, including α-KG , fumarate, malate and citrate. SK-Hep-1 cells expressing empty vector or PCK1 were cultured in media containing 0.1 mM of glucose for 5 h in the absence or presence of dimethyl-α-KG (5 mM). Metabolites were measured by GC-MS. α-KG level was not detected in cells without addition of exogenous dimethyl-α-KG due to its low level and insufficient detection sensitivity. Values represent mean abundance of indicated metabolites ± SD of quintuplicate samples (***$p < 0.001$). ND, not detected.



Supplementary Figure S5. Increased PCK1 expression suppressed liver tumorigenesis. (a) The expression levels of PCK1 were reduced in YAP(5SA) induced liver tumors in 4 mice. T: liver tumor; N: adjacent normal liver tissue. (b) The expression of flag-PCK1 and myc-YAP in mouse liver was detected at 24 h post hydrodynamic injection by Western blotting.